



Quality Antibody Production Since 1960

Pocono Rabbit Farm & Laboratory, Inc.,
**PRF&L Standard Protocols for Custom
Polyclonal Production in Rabbits**

STANDARD PROTOCOL for PROTEIN and PEPTIDE ANTIGENS

Day 0	Large (10-15ml sera) or Small (3-5ml sera) Preimmune Bleed. Preimmune will be stored here at -80 degrees C until the first immune bleed. Inject 100-200ug into the popliteal lymph nodes or intradermal, or both, in Complete Freund's Adjuvant (CFA). We will inject intradermal unless you specify lymph node. We will inject intradermal and subcutaneous if the concentration of antigen is less than 0.3mg/ml. Acrylamide gel can only be injected subcutaneous.
Day 14	Inject 50-100ug intradermal in Incomplete Freund's Adjuvant (IFA)
Day 28	Inject 50-100ug subcutaneous or intradermal (if we injected lymph node on Day 0) with IFA.
Day 42	Small Bleed (3-5ml sera) The Monday following Day 42 we will ship via Federal Express the preimmune bleed and this first test bleed on dry ice. It is then up to the investigator to test the serum, determine the quality of antibodies, and let us know if any changes are necessary in the protocol. PRF&L now offers <u>antibody titration by ELISA</u> and can now check titers for you. There is an additional charge for this service.
Day 56	Inject 25-50ug subcutaneous with IFA
Day 70	Small Bleed (3-5ml sera) We will do small bleeds every 28 days unless you specify. The Monday following Day 70 we will ship via Federal Express this small bleed. It is up to the investigator to test the serum, determine the quality of antibodies, and let us know if any changes are necessary in the protocol. PRF&L now offers <u>antibody titration by ELISA</u> and can now check titers for you. There is an additional charge for this service.
After Day 70	See Continuing your Protocol below.

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Ruben EXHIBIT 2023
 Ruben v. Wiley et al.
 Interference No. 105,077
 RX 2023

2023



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We will continue give boosts every four weeks with 25-50ug of antigen subcutaneous with IFA. We will do a small bleed two weeks after each boost and send to you the following Monday. We will continue to boost and bleed every four weeks until you contact us. Keep in mind that you have several other options depending on your needs and the success of the project.

When the antisera contains sufficient antibody titer, we can start production bleeds. We can do production bleeds every seven days. Please contact us if you want to change the bleeding and/or boosting schedule. You may also request that we not do any boosts after the initial immunization scheme outlined above since some antigens and some animals may respond negatively to boosts during the period of peak antibody activity. Boosts may be required only when titer drops. It is up to the investigator to decide and let us know if we should discontinue boosts as our standard schedule provides for boosts every 28 days.

The volume of serum collected for large or production bleeds depends on the weight and the general health of the Rabbit. We generally take 25-40ml of blood yielding 13 to 20ml of serum.

Our experience shows that the peak antibody titer occurs at an average of 120 days, however we have experienced the onset of peak activity as early as six weeks and as long as a year. If results are not as you expect, please consult with us and we can make recommendations on how to change your protocol.

When you have collected enough antisera, you will need to contact us to terminate the project. You may request that we perform an exsanguination (terminal bleed) or if no additional sera is required, the animal(s) will be humanely euthanized (discard). When you instruct us to terminate your animal(s), you must tell us if you want exsanguination or discard, species, and the animal(s) number. Written confirmation by email or fax may be requested for terminal procedures.

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